Vaccination with Human Hookworm Vaccine "*Necator americanus* Aspartic Protease-1 M74" Generates Neutralizing Antibodies and a Potent Immune Response in BALB/c Mice

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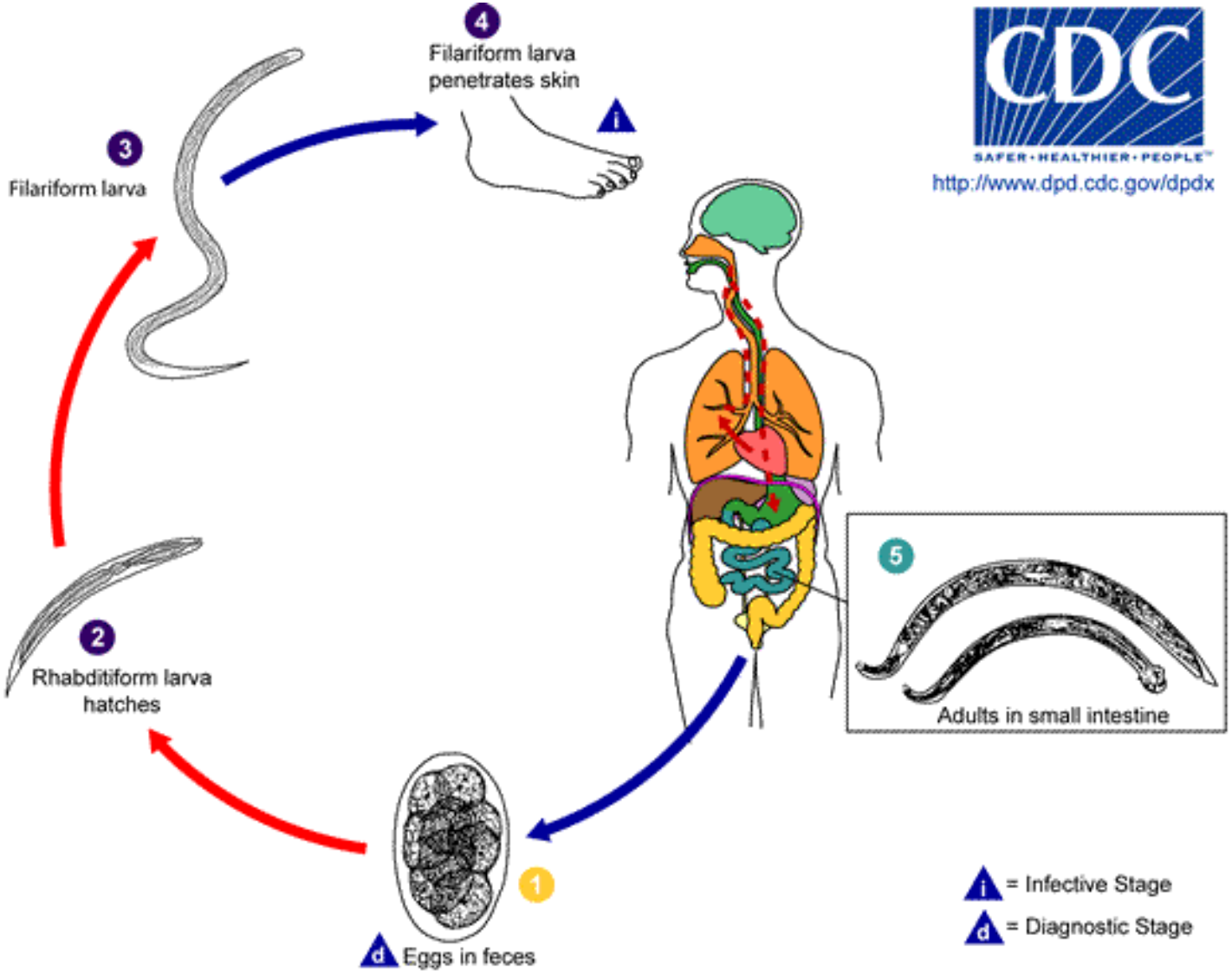
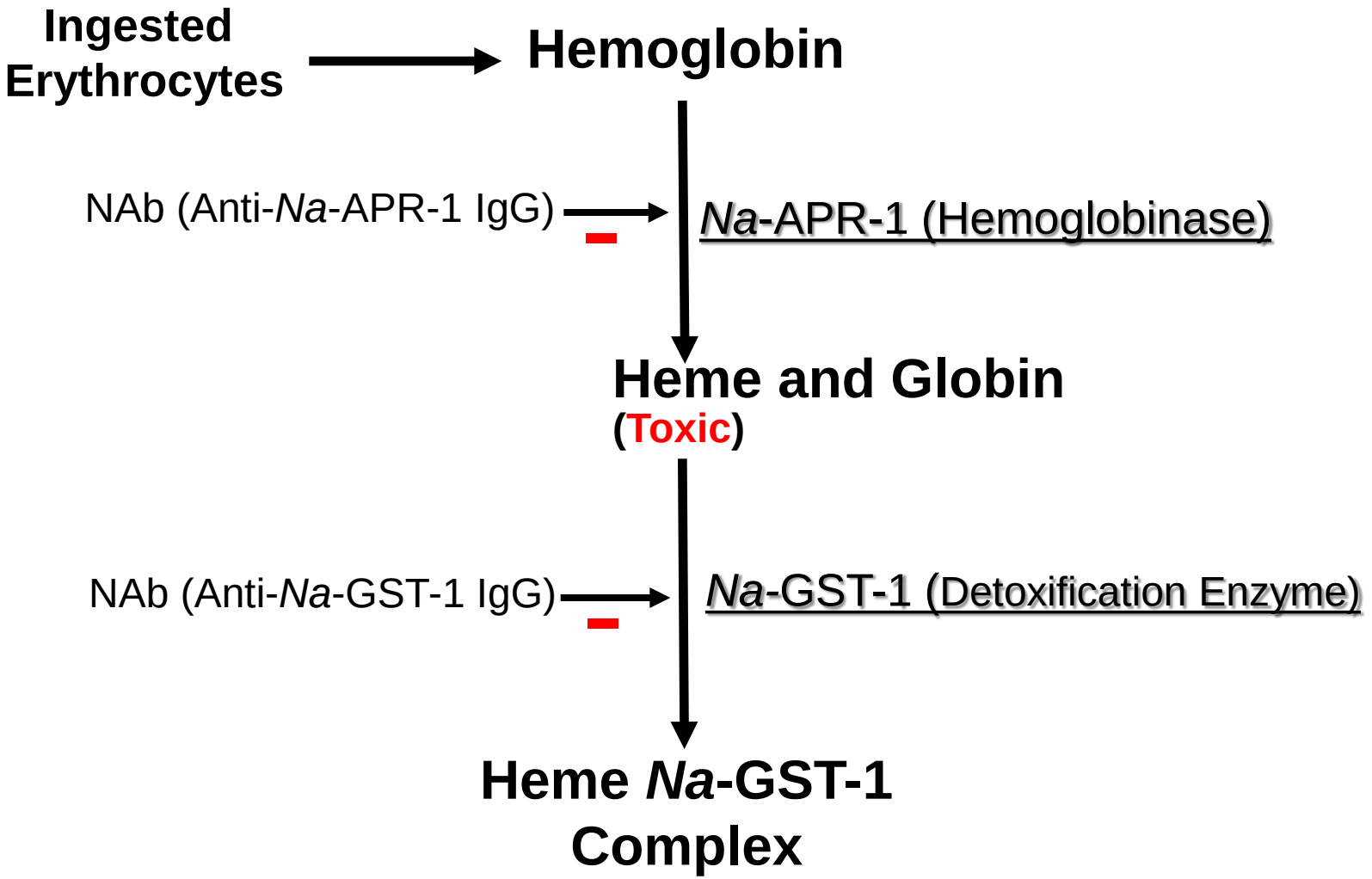
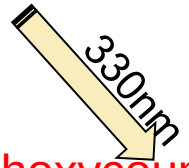
Life Cycle of *Necator americanus* Human Hookworm



Necator americanus Degradation of Host Hemoglobin and Vaccine Targets



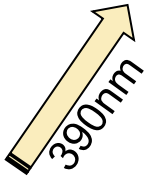
Method† (MOD=Fluorescence)



7-methoxycoumarin-4-acetyl-GKPILFFRLK(dinitrophenyl)-d-Arg-amide

(fluorophor) (quencher)

Cleavage site



7-methoxycoumarin-4-acetyl-GKPILF FRLK(dinitrophenyl)-d-Arg-amide

(fluorophor) (quencher)

***Na*-APR-1wt**

7-methoxycoumarin-4-acetyl-GKPILFFRLK(dinitrophenyl)-d-Arg-amide

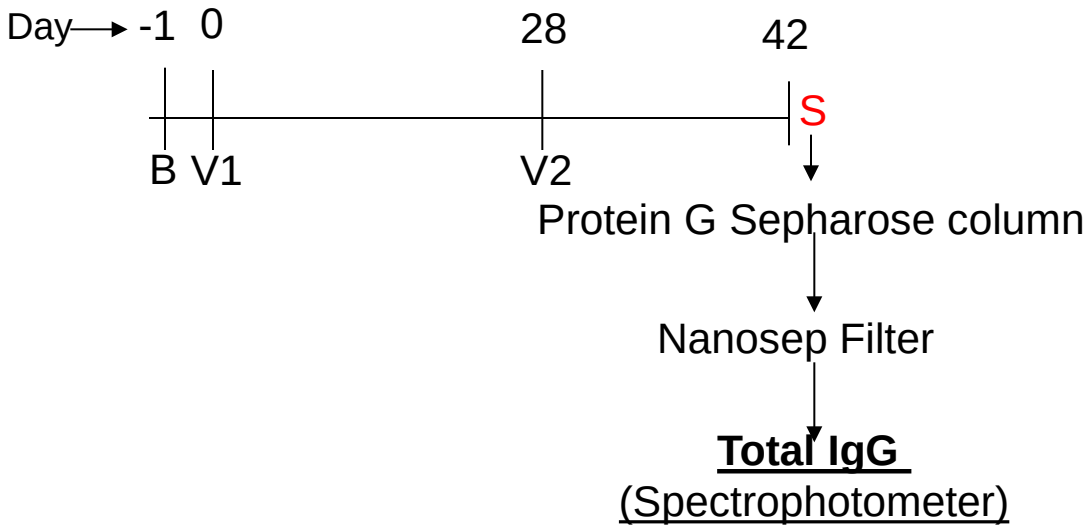
(fluorophor) X (quencher)

***Na*-APR-1wt**

Anti-*Na*-APR-1 M74 IgG

†**Reaction Buffer - 50 mM Sodium Acetate (pH = 6.0).**

Steps for Generation and Purification of Polyclonal Mouse IgG†



B= Bleed
V= Vaccination (subcutaneously)
S= Sacrificed

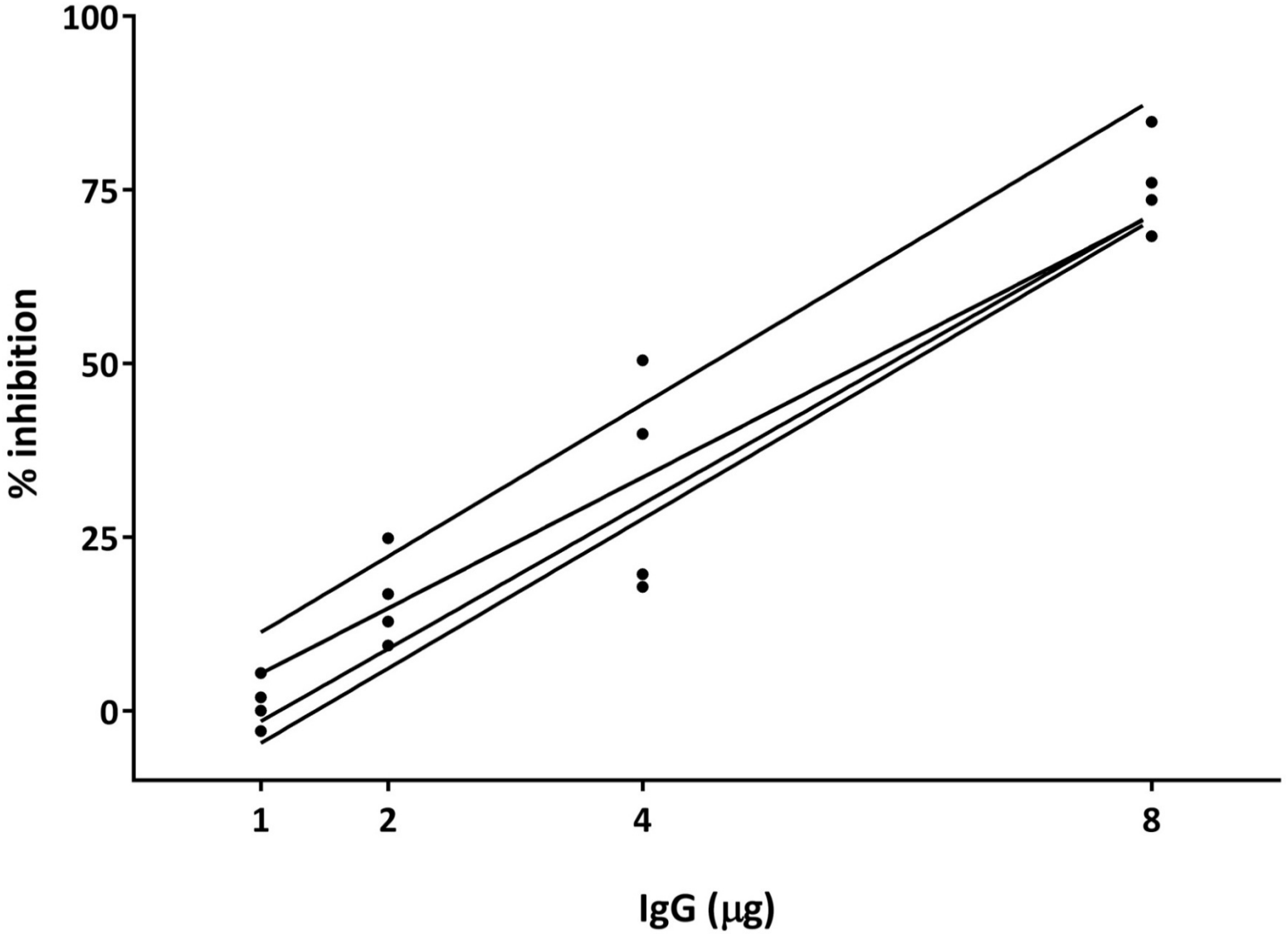
†50 BALB/c mice vaccinated twice with 9.33µg *Na*-APR-1 M74 + 74.64µg Alhydrogel®.

Neutralizing Capacity of purified IgG with 250 ng of *Na*-APR-1wt and 1µM Cathepsin-D Substrate in 50mM Sodium Acetate, pH 6.0.

	IgG (µg)	Operators	% Inhibition*	Intra-plate %CV	Inter Operator %CV	All Runs %CV
Run 1	5	1	48.93	4.86	9.81	14.57
		2	42.58	6.98		
Run 2		1	60.44	19.39	10.22	
		2	52.29	7.68		

*RFU of the purified IgG compared to RFU of Negative IgG (commercial mouse IgG).
Note: All the components were incubated at 37°C for 30 Min. RFU=Relative fluorescence Units.

Neutralizing Capacity of 8, 4, 2 and 1 µg of IgG with 250ng of *Na*-APR-1wt

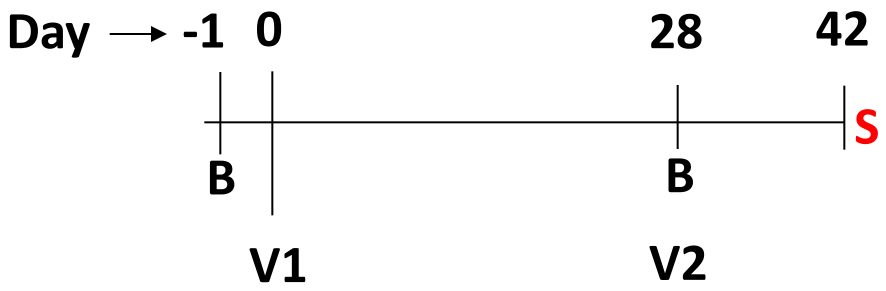


Note: All the components were incubated at 37°C for 30 Min.
RFU of Positive IgG compared to RFU of Negative IgG (commercial mouse IgG).
RFU=Relative fluorescence Units.

Potency Testing of *Na*-APR-1 M74 Clinical Drug Product†

BALB/c mice immunized Subcutaneously with *Na*-APR-1/Alhydrogel® at time 1, 7 months post manufacture. (10 mice in 1 group)

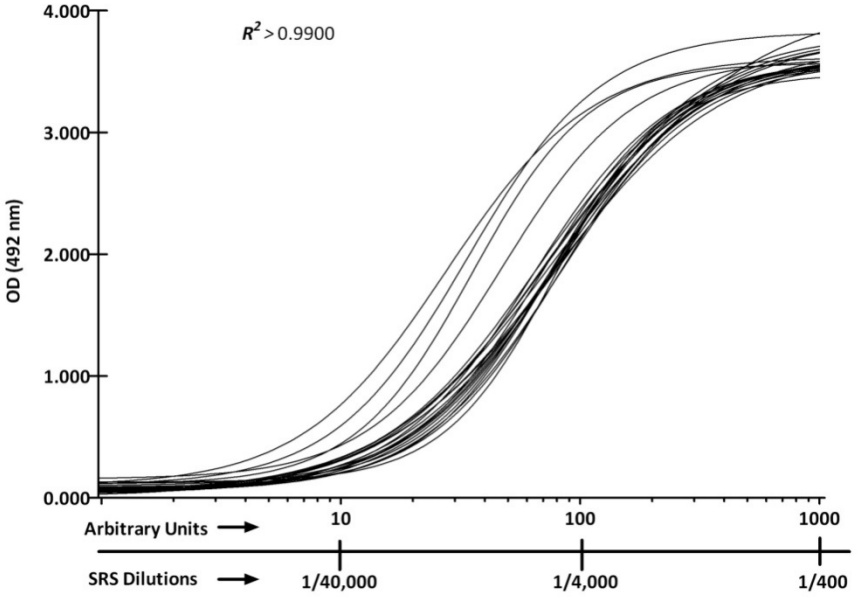
Na-APR-1 (µg)	Alhydrogel® (µg)	Volume (ml)
N/A	400	0.5
50.00	N/A	0.5
50.00	400.00	0.5
28.57	228.56	0.2857
16.33	130.64	0.1633
9.33	74.64	0.0933
5.33	42.64	0.0533
3.05	24.40	0.0305
1.74	13.92	0.0174
0.99	7.92	0.0099

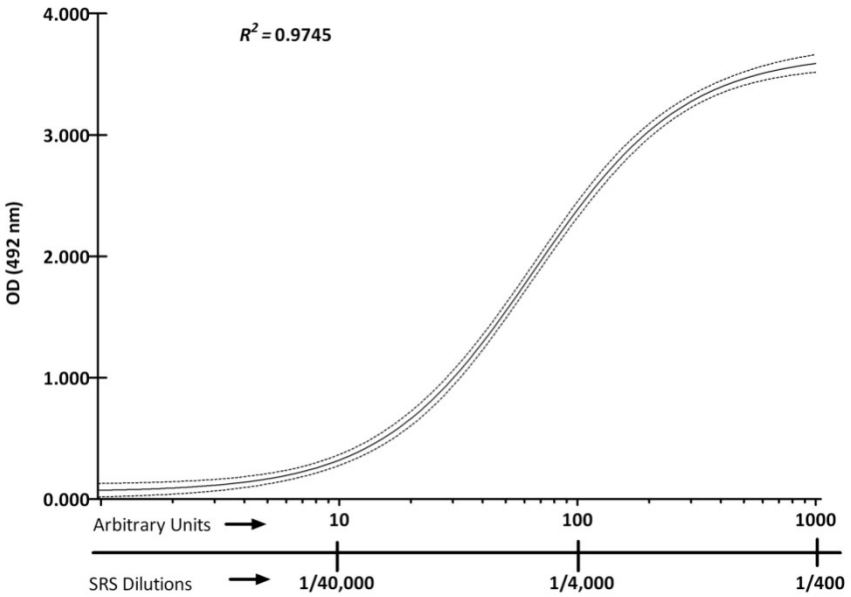


B= Bleed
V= Vaccination
S= Sacrificed

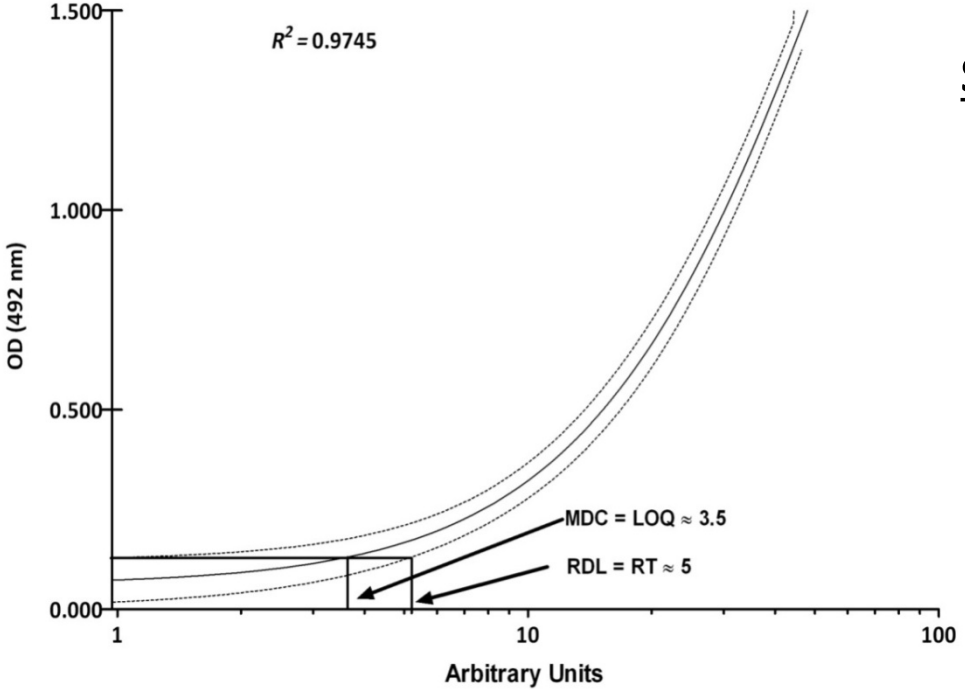
†0.1mg/mL *Na*-APR-1 (M74) and 0.8mg/mL Alhydrogel® in 150 mM NaCl/10mM imidazole/0.3% Empigen BB

Standard Calibration Curves (22 curves from 22 Anti *Na*-APR-1 M74 IgG ELISA Plates)





LOQ and RT



Standard Reference Serum

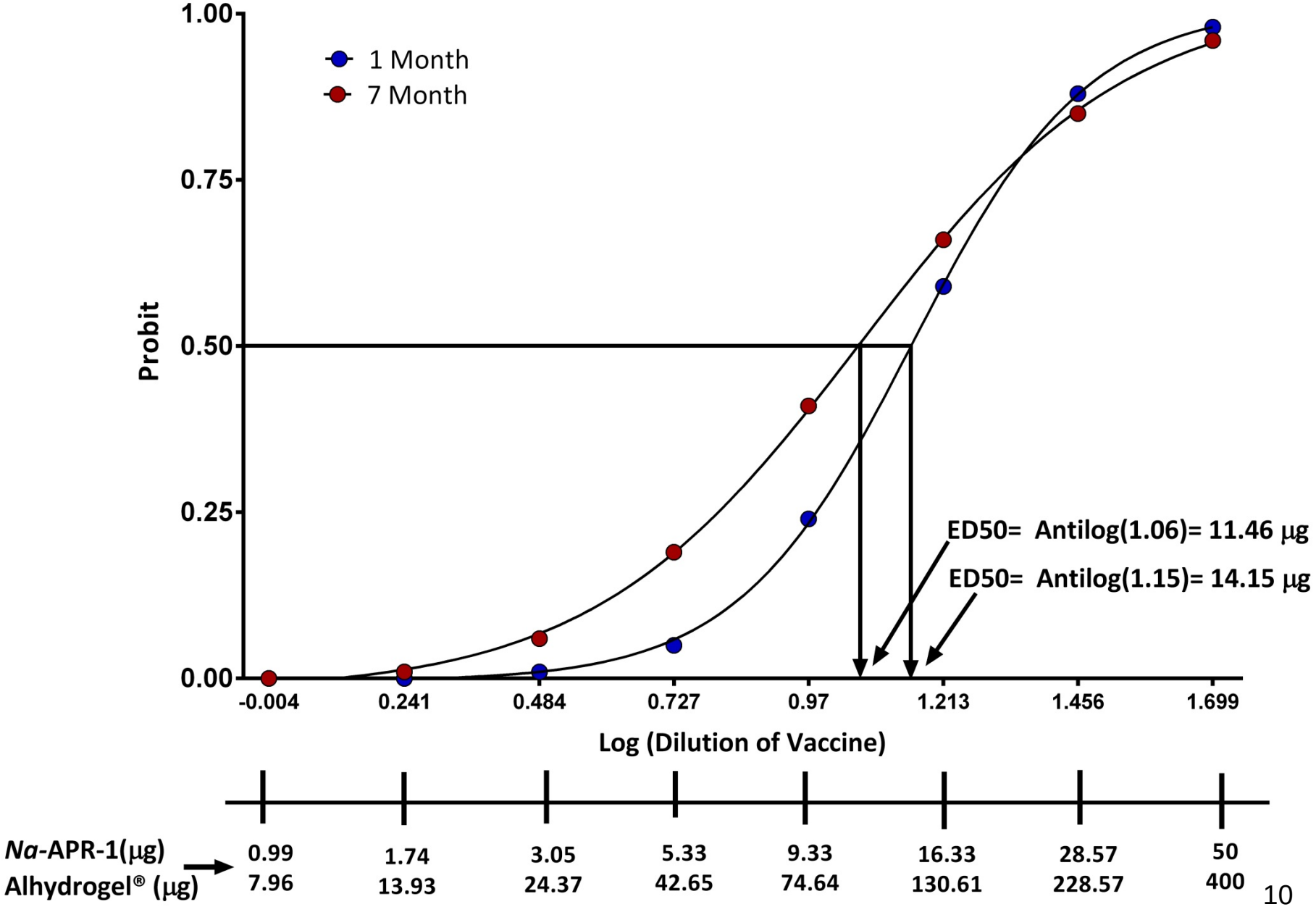
B= Bleed
V= Vaccination (subcutaneously)
S= Sacrificed

9.33µg *Na*-APR-1 M74 + 74.64µg Alhydrogel®

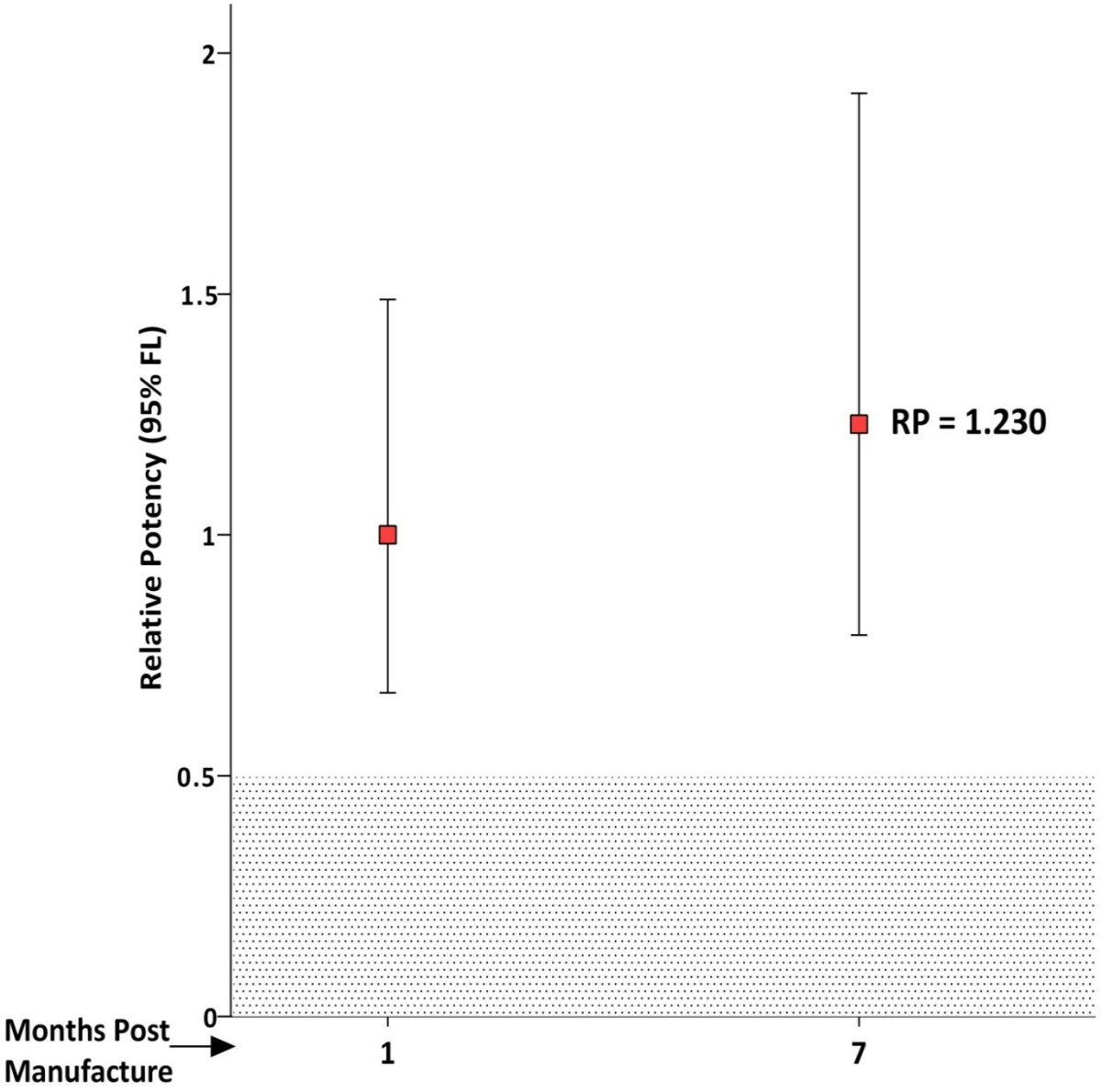
Responders on Study Day 28 Post-Prime Vaccination

Fractional Doses (µg)		Responders	
		Month	
Na-APR-1 M74	Alhydrogel®	1	7
N/A	400	0	0
50.00	N/A	0	0
50.00	400.00	9	10
28.57	228.56	10	6
16.33	130.64	6	7
9.33	74.64	3	8
5.33	42.64	0	1
3.05	24.40	0	0
1.74	13.92	0	0
0.99	7.92	0	0
ED50 (Effective Dose 50) (µg)		14.15	11.46
95% Fiducial Limits (µg)		10.47 -- 18.93	4.86 -- 27.42

ED50 on Study Day 28 Post-Prime Vaccination



Relative Potency (RP) of *Na*-APR-1 M74 Vaccine



Summary

- Five microgram of purified IgG from BALB/c mice vaccinated with 9.33 µg *Na*-APR-1 M74 and 76.64 µg Alhydrogel® neutralized 51.06% of the enzymatic activity of 250 ng of *Na*-APR-1wt

- An excellent dose response (% inhibition vs IgG) was observed

- The standard reference serum generated an excellent standard calibration curves (SCCs) as well as an excellent global standard calibration curve (GSCC) using an Anti-*Na*-APR-1 M74 IgG ELISA

- *Na*-APR-1 M74 vaccine generated a potent immune response in Balb/c as evident by the generation of ED50 at time 1 and 7 month post-manufacture

- *Na*-APR-1 M74 vaccine became 1.23 times more potent at time 7 month when compared to its potency at time 1 month post-manufacture

Acknowledgments

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